CLAIMS

1. PURIFYING PROCESS OF SOLUBLE PROTEINS OF THE *L. OBLIQUA* BRISTLES THROUGH PROTHROMBIN ACTIVATION, characterized for containing the following stages:

a) Homogenize *L. obliqua* bristles in phosphate-buffered saline (PBS), pH 7.4-8.0, centrifuge at 4° to 10° C by 2500xg from 30 to 60 minutes to obtain a crude extract from the prothrombin activator;

b) Purify the prothrombin activator from 50 to 200 mg of whole protein from 2 to 10 ml of crude extract through gel-filtration chromatography in Sephadex G-75 resin. Elute in 20 to 50 mM Tris-HCL buffer containing NaCL 50 to 100 mM and benzamidine 2 to 5 mM, pH 7.4 to 8.0 with flow of 1,0 ml/h;

c) Collect fractions from 1 to 3 ml and monitor the chromatography protein profile by UV absorbency in 280 nm;

d) Activate the pròthrombin using the protein peaks obtained and the S-2238 colorimetric substrate, specific for thrombin;

e) Obtain the peak PII presenting the activation of prothrombin;

f) Submit the active fraction obtained to a reverse-phase chromatography in column C4 using HPLC analytic system. Use as solvents: - A: 0,1% TFA in water (balanced) and B: solvent A and acetonitrile in a proportion of 1:9

(elution) and proceed the protein detection of 214 to 280 nm in UV monitor;

g) Collect fractions of 0.5 - 1.0 ml and lyophilize them immediately for eliminating acetonitrile;

h) Suspend again the lyophilized samples in 20 to 50 mM Tris-HCL buffer containing 50 to 100 mM NaCL, pH 7.4 to 8.0;

i) Test activation of prothrombin activator of the fractions as described in item d);

j) The active peak in fractions is eluted between 42 to 44% of solvent B;

k) Submit the active fraction again to a chromatography as described in item (f) using a gradient between 20 - 80% of solvent B, during 20 minutes;

l) Repeat the stages from (f) through (j);

m) Submit the purified material to an electrophoresis in polyacrilamide gel containing SDS for homogeneity evaluation. This gel could be stained by Coomassie brilliant blue;

n) Evaluate the final protein concentration by protein assay using colorimetric methods or Absorbency in 280 nm in order to obtain the prothrombin activator;

2. PROCESS in accordance with claim 1, characterized by using in stage (b) the following solvents for elution: solvent A: 0,1% TFA in water and solvent B: solvent A and acetonitrile in a proportion of 1:9.

3. PROCESS in accordance with claim 1 characterized by using the HPLC analytic system in stage (f) produced by Merck-Hitachi (D-2500 model) and the monitor of stage (g) produced by Shimadzu UV (SPD-6AV model);

4. PROCESS in accordance with claim 1 characterized by using the HPLC purification in the stage (f) using a gradient of 35-50% of solvent B;

5. PROCESS FOR PARTIAL DETERMINATION OF THE AMINO ACIDS SEQUENCE OF THE PROTHROMBIN ACTIVATOR characterized by degrading 500 - 1000 pM of purified protein with 10 pmol of trypsin in 100mM Tris-HCl, pH 8.0 containing 0.02% of $CaCl_2$ during 18 hours at 37°C stopping the reaction with 15 % (v /v) of formic acid;

6. PROCESS in accordance with claim 5 characterized by separating through HPLC, the fragments obtained in the column C4, eluted with solvents 0,1% of TFA in water (solvent A) and acetonitrile: solvent A (9:1) (solvent B);

7. PROCESS in accordance with claim 6 characterized by using a gradient of 0-100% of solvent B with flow of 1.0 ml/min during 30min for the HPLC separation;

8. PROCESS in accordance with claim 7 characterized by determining sequence of four internal peptides and the N-terminal sequence;

9. PROCESS in accordance with claim 8 characterized by N-terminal portion containing 46 residues of amino acids (DVVIDGACPDMKAVSKFDMNAYQGTWYEIKKFPVANEANGDCGSV E) and the internal peptides fragments being: Fragments I (KSHVYTVPFGA); Fragment II (KSNQHRVNIWILSRTK); Fragment III (VRAGHVE) and Fragment IV (FDQSKFVETDFSEKACFF);

10. PROCESS in accordance with claim 9, characterized by the sequence obtained of about 15% of the whole protein considering 69KDa its molecular mass;

11. PROCESS FOR DETERMINATION OF THE PROTHROMBIN ACTIVATION OF FRACTION II, characterized by containing the following stages:

a) Pre-incubate 15 to 300nM of the purified fraction during 10 minutes at 37° C with 90 pM of prothrombin using 5mM of $CaCl_2$ for final volume of 500µL using 50mM Tris-HCl, 100mM NaCl, pH 8 as well as 150 mM of imidazol;

b) Add 40 µM of chromogenic substrate S-2238 (H-D-phenylalanyl-L-pipicolyl-L-arginine-p-nitroanilide dihydrochloride), to the incubation mixture and evaluate spectrophotometrically the chromogenic substrate hydrolysis through 405 nm during 10 minutes;

12. N-TERMINAL SEQUENCE AND SEQUENCE OF INTERNAL FRAGMENTS OF THE PROTHROMBIN ACTIVATOR FRACTION characterized by containing 46 residues of amino acids (DVVIDGACPDMKAVSKFDMNAYQGTWYEIKKFPVANEANGDCGSV E) in the N-terminal portion and the internal peptide fragments are: - Fragment I (KSHVYTVPFGA); Fragment II (KSNQHRVNIWILSRTK); Fragment III (VRAGHVE) and Fragment IV (FDQSKFVETDFSEKACFF) and the sequence obtained corresponds to about 15% of the whole protein with molecular mass of 69 KDa;

13. PROTHROMBIN ACTIVATOR was obtained in accordance with the process of claims from 1 through 11, characterized by containing the following structure: The purified protein is characterized as a serine protease which hydrolyses the prothrombin generating Fragments 1, 2 and thrombin as showed in the figures;

14. THE UTILIZATION OF THE PROTHROMBIN ACTIVATOR of claim 13, characterized by enabling to be using prothrombin activator as a dysfibrinogening agent in prothrombotic state patients;

15. THE UTILIZATION OF THE PROTHROMBIN ACTIVATOR of claim 13, characterized by enabling to be used for producing diagnosis kits for detecting

plasmas prothrombin in hemmorhragic state patients.